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EXAMINER	
SWITZER, JULIET CAROLINE	
ART UNIT	PAPER NUMBER
1634	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/856,333	BERLIN, KATHRIN	
	Examiner	Art Unit	
	Juliet C Switzer	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 October 2004*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9,11-16,18-24,26,27 and 30* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9,11-16,18-24,26,27 and 30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. This action is written in response to applicant's correspondence submitted 10/18/04. Claims 1, 2, 3, 4, 5, 6, 7, 9, 11, 16, 18, 26, and 27 have been amended. Claims 10, 17, 25, 28, and 29 have been cancelled, and claim 30 has been added. Claims 1-9, 11-16, 18-24, 26-27, and 30 are pending. Applicant's amendments and arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections not reiterated in this action have been withdrawn. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action. **This action is FINAL.**

Claim Rejections - 35 USC § 112

2. The rejections of claims 2, 3, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 25, and 28 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention are WITHDRAWN in view of applicant's amendments to the claims.

3. The rejections of claims 16, 18, 26 and 27 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement are WITHDRAWN in view of applicant's amendments to the claims.

4. The rejection of claim 6 under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement is WITHDRAWN in view of applicant's amendment to the claim and applicant's explanation provided on pages 14-15 of the response.

Claim Rejections - 35 USC § 103

5. Claims 1-6, 8, 11, 18-25, 28, and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rice *et al.* (Oncogene (1998) 17, 1807-1812) in view of Gifford (US 5750335).

Rice *et al.* teach a method for identifying 5-methylcytosine positions in a sample genomic DNA, said method comprising the steps of:

(a) chemically treating a sample genomic DNA obtained from at least one cell in such a way that cytosine and 5-methylcytosine react differently and from products with different base pairing behavior (p. 1811, treatment with sodium bisulfite);

(b) amplifying by means of a polymerase reaction a segment of the genomic DNA obtained in step (a) (p. 1811, second column);

(c) performing steps (a) and (b) on a reference genomic DNA (Figure 3, methylation was determined for eight different cell types);

Rice *et al.* utilize chemical treatment with sodium bisulfite in a method to identify the location of methylated cytosines in genomic DNA. Treatment with bisulfite results in the conversion of unmethylated cytosine residues to uracil, while methylated cytosine residues remain unchanged. Thus, in a sample with unmethylated sequence (for example the HMEC) there would be no change in sequence, but in a sample with high levels of methylation, after PCR there would be thymines where the methylated cytosines previously were located. Rice *et al.* effectively introduce mutations nucleic acid sequences via the treatment with sodium bisulfite. Rice *et al.* utilize a sequencing method to determine the methylation positions after amplification of the sequences.

With regard to claim 2, in the method taught by Rice *et al.* positions which are variable between different cell lines are identified (see figure 3).

With regard to claim 3, Rice *et al.* utilize a bisulfite to treat the genomic DNA.

With regard to claim 4, Rice *et al.* jointly amplify genomic DNA from several cells, as they necessarily isolated DNA from more than one cell for each cell line.

With regard to claim 5, Rice *et al.* separately amplified the DNA from several cell lines, and then treated them all with bisulfite.

With regard to claim 8, Rice *et al.* test unmethylated cell lines (p. 1807, second column; Figure 3).

With regard to claim 18, a nucleotide sequence is considered a "chemical function" that enables a PCR product to be immobilized on a surface. Therefore, the PCR carried out by Rice *et al.* necessarily uses a primer that enables the polymerase reaction to be immobilized on a surface. The claim does not actually require an immobilization step.

Rice *et al.* do not form heteroduplexes from the amplified products for the comparison of a test and reference sample.

Gifford teaches a method for identifying sequence differences between two nucleic acids that comprises the steps of:

(d) forming heteroduplexes from two different nucleic acid samples (Col. 3, lines 40-50);

(e) introducing a detectable label into the heteroduplexes of step (d) by means of a reaction which is specific for non-complementary base pairs (Col. 4, lines 15-20), and

(f) determining the position of 5-methylcytosine in the sample genomic DNA based on the presence and position of the detectable label (Col. 4, lines 5-10, 20-25).

Gifford specifically teaches comparing a sample (patient) nucleic acid fragment with a control (normal) nucleic acid fragment (Figure 2).

With regard to claims 4 and 5, Gifford teaches that a test or reference nucleic acid may include monoclonal or polyclonal cell lines (Col. 9, 22-25).

With regard to claims 19, Gifford teaches that the reference or test nucleic acids may be immobilized to a solid surface (Col. 5, lines 1-5; col. 13, lines 1-5). With regard to claim 20, Gifford teaches that "different" reference nucleic acids may be immobilized on a solid surface at different spots, which are considered different reaction vessels (Col. 5, lines 1-5). Further, Gifford teaches the transfer of the amplified products to different vessels (affinity columns or affinity matrix) for purification of the heteroduplexes wherein the products are coupled to a solid support (column 5, lines 47-56).

With regard to claim 21 and 22, Gifford teaches an using an enzyme that forms a complex with a non-complementary base pair (Col. 4, lines 10-20), specifically teaching MutS (Col. 7, line 22).

With regard to claim 23, Gifford teaches a method wherein the enzyme bears a label by which a complex can be displayed (Col. 15, lines 65-67).

With regard to claim 24, Gifford teaches that the label is a fluorescence label (Col. 15, lines 65-66).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the methylation detection method taught by Rice *et al.* so as to have utilized the mutation detection methods taught by Gifford. One would have been motivated to utilize the methods taught by Gifford in order to achieve the express benefits of the

methods taught by Gifford which include achieving "rapid and accurate genetic screening and diagnosis by comparing two nucleic acids for differences in their sequences...to locate previously unknown mutations of a nucleotide sequence, and to identify the sequence itself, where the nature and position of the mutation within a region of the genome is unknown, and where the location of the region itself is unknown (Col. 3, lines 25-40)." With regard to claim 6, the result required in claim 6 would have been a necessary property of the practice of the assay taught by Rice *et al.* in view of Gifford. Namely, where there was differential methylation between the sample genomic DNA and any of the reference DNA's taught by Rice *et al.,* when these were subjected to heteroduplex analysis as taught by Gifford erroneous base pairings would have been produced at the positions at which 5-methylcytosine was located in the sample genomic DNA but not in the reference genomic DNA.

6. Claims 9, 11, 12, 13, 14, 15, 16, 17, 26, 27, and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rice *et al.* in view of Gifford as applied to claims 1-5, 7-8, 11, 18-25, 28, and 29 above, and further in view of Koster *et al.* (US 6428955).

The teachings of Rice *et al.* in view of Gifford are applied herein as applied in the previous rejection. In the method taught by Rice *et al.* in view of Gifford, sodium bisulfite is used which results in the modification of unmethylated cytosines, and therefore mismatches would occur at positions where cytosine was located in the genomic DNA. Rice *et al.* in view of Gifford do not teach a method in which the heteroduplex is detected by cleavage of the heteroduplex molecule or in which mass spectrometry is used to analyze the size of the DNA fragments.

With regard to claims 11-14, Koster *et al.* teach methods for analyzing the size of nucleic acid fragments using mass spectrometry, specifically teaching the use of MALDI-TOF and ESI, (Col. 18, line 66-Col. 19, line 11).

With regard to claim 15, which requires that the nucleic acids in step (e) are "adapted" to the performance capacity of the mass spectrometer, Koster *et al.* teach utilizing a variety of PCR amplification methods to obtain PCR products that they analyze using the mass spec (See examples 14-15, for example). With regard to claim 16, Koster *et al.* teach utilizing nested PCR to amplify products for detection (Examples 5 and 14, for example), a method which uses primers that are set stepwise along the DNA with respect to the inner and outer pairs of primers and produce a series of amplification products, at least one which is within the mass range detectable by means of mass spectrometry. These PCR primers are considered to be "set stepwise" since they amplify two differently sized products where on is a size "step" down from the other.

Claim 26 differs from claim 1 in that in step (b) the PCR primer is fluorescently labeled and provided with a chemical function thereby enabling the immobilization of the amplificate on the surface, step (e) utilizes a chemical mismatch cleavage methodology, and step (f) utilizes mass spectrometry, whereby in step (g) the presence or presence and position of the 5-methycytosine within the genomic DNA is deduced from the length of the cleaved nucleic acids. Claim 27 is similar to claim 26 but requires that a detectable label is introduced into the heteroduplex by an enzymatic reaction which is specific for non-complementary base pairs. This limitation is provided in the methods taught by Rice *et al.* in view of Gifford.

Likewise, newly added claim 30 is similar to claim 26, and thus also similar to claim 1. Steps (a)-(d) of claim 30 are similar to those of claim 1. Unlike claim 1, however, claim 30 requires that the heteroduplexes of step (d) are cleaved by a chemical mismatch cleavage reaction. Claim 9 depends from claim 30 and requires that the nucleic acid backbone of the heteroduplex is specifically cleaved at the non-complementarily base paired positions by an enzymatic means.

Koster *et al.* teaches a method in which a heteroduplex is cleaved by an agent that cleaves the unhybridized portion so that a mismatch results in two products and then detecting these by mass spectrometry to detect the presence of the mismatch (Col. 5, lines 30-40; Col. 23, lines 25-40). Koster *et al.* further teach primers that are labeled with biotin (a means for immobilizing an amplificate on a surface; col. 35, for example) and primers that are labeled with a radioactive label and oligonucleotides that are fluorescently labeled (Col. 49, for example). In addition, as noted previously in this office action, any nucleic acid sequence itself is considered a "chemical function" that would enable the immobilization of the amplificate on a surface. Claims 26 and 27 never actually require the immobilization of the amplificate on a surface, only that such immobilization is "enabled." Nonetheless, Koster further teach methods in which the sequence to be detected is immobilized to a solid support by means of hybridization (Col. 3, lines 60-67).

It would have been prima facie obvious to one of ordinary skill in the art to have modified the method taught by Rice *et al.* in view of Gifford *et al.* so as to have used the amplification and detection methods taught by Koster *et al.* One would have been motivated to use mass spectrometry as a means for detection of nucleic acid fragments in order to take advantage of the express benefits of such a method as taught by Koster *et al.*, who state "the

processes of the invention provide for increased accuracy and reliability of nucleic acid detection by mass spectrometry (Col. 5, lines 62-65). Furthermore, it would have been prima facie obvious to have utilized fluorescently labeled primers in place of the radioactively labeled primers taught by Koster *et al.* in order to have provided an alternative labeling method that is safer to use as opposed to using radioactivity in the laboratory.

7. Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Rice *et al.* in view of Gifford as applied to claims 1-5, 7-8, 11, 18-25, 28, and 29 above, and further in view of Nazarenko *et al.* (US 6090552).

The teachings of Rice *et al.* in view of Gifford are applied herein as applied in the previous rejection. Rice *et al.* in view of Gifford do not teach methods wherein the reference DNA is methylated at all CpG positions.

However, the inclusion of a methylated control in an assay for the determination of methylation would have been routine at the time the invention was made. Nazarenko *et al.* teach methods for detecting methylation in samples, and teach the inclusion of methylated control nucleic acids in these assays and in kits for performing these assays (see Col. 37-38 and 49-50, for example). Thus, It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have included in the assays taught by Rice *et al.* in view of Nazarenko *et al.* a control which is methylated at all CpG positions in order to have had a standard for comparison of all results against those obtained with a methylated control.

Response to Remarks

With regard to the reiterated 103 rejections, which are modified to address the newly added and amended claims, applicant first discusses the benefits of the instant invention at pages

18-19. Applicant points out that Rice *et al.* rely on sequencing the sample in order to determine a methylation pattern. It is noted that this is a piecemeal analysis of only Rice *et al.*, which does not address the totality of the rejection. The rejection specifically discusses the modification of the methods taught by Rice *et al.* so as to use the heteroduplex analysis as taught by Gifford for the determination of methylation patterns. Nonetheless, it is further noted that the instant claims do not exclude the use of a sequencing step, for example, after fragments containing heteroduplexes are identified, as the claims are broadly drawn using "comprising" language.

8. Applicant argues that nothing in Gifford teaches or suggests step (f) in claim 1. Gifford does teach identifying the position of mismatched nucleotides, as is cited in the office action. The statement that Gifford specifically teaches detecting methylation is a typographical error. Clearly, the examiner pointed to the porition of Gifford where Gifford teaches identifying the position of a mismatch between two sequences. Rice *et al.* provides a method for modifying genomic DNA at positions of methylation (i.e. introducing mismatches using bisulfite). Thus, as stated in the rejection, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have used the methods taught by Herman to identify mismatches among the sequences being inquiried by Hall *et al.* Thus, the identification of these mismatches using the method taught by Gifford would have necessarily resulted in the determination of the position of the 5-methylcytosines in the genomic DNA, as the mismatched sequences among the different cell lines would represent portions of the genome that are differentially methylated from one genomic DNA sample to another. Applicant further argues that it would have required hindsight reconstruction to arrive at the instant invention. However, as discussed in the rejection, Gifford teaches a number of different reasons why one would have

been motivated to have utilized the methods taught therein for the detection of sequence differences between two sequences. Rice *et al.* undertake a method to introduce sequence differences where methylation is differential between two sequences. Thus, in view of the teachings of the benefits of the methodology taught by Gifford, one would have been motivated to utilize heteroduplex analysis as taught by Gifford in order to have attained the benefits taught by Gifford. In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). In this case, the benefits of using the method taught by Gifford are clearly taught by Griffin. Thus, as stated in the rejection, it would have been obvious to have used the methods taught by Gifford to detect the mismatches between sequences introduced by Rice et al. into the sample and reference genomic DNA molecules. The rejections are MAINTAINED and applied to the newly added and amended claims.

Conclusion

9. No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Juliet C Switzer whose telephone number is (571) 272-0753. The examiner can normally be reached on Monday through Friday, from 9:00 AM until 4:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, W. Gary Jones can be reached by calling (571) 272-0745.

The fax phone numbers for the organization where this application or proceeding is assigned are (703) 872-9306. Any inquiry of a general nature or relating to the status of this

application or proceeding should be directed to the receptionist whose telephone number is (571)272-0507.

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Juliet C. Switzer
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Art Unit 1634

January 7, 2005